The Packaging of Ova in the Egg Cases of *Aplysia californica*

BY

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(5 Text figures)

INTRODUCTION

THE MARINE MOLLUSK *Aplysia* is a prolific egg-layer. Large animals are known to lay about 5×10^8 eggs during the several months of their reproductive life (MACGINITIE, 1934). Egg-laying behavior has other interesting features. Animals generally do not lay small amounts of eggs continuously, but deposit a large number in all-or-none episodes (KUPFERMANN, 1970; KANDEL, 1976). In addition, egg-laying is one of the rare examples of a behavior in which the neurobiological substrate underlying it is relatively well understood (for reviews see KUPFERMANN, 1972; KANDEL, *op. cit.*). A hormone controlling egg-laying is released by 2 cell clusters called bag cells; each cluster contains about 400 neuroendocrine cells. The active egg-laying hormone released by the bag cells is a polypeptide with a molecular weight of approximately 6 000 dalton (TOEVS & BRACKENBURY, 1969; GAINER *et al.*, 1974; ARCH, 1972). A 25 000 dalton precursor polypeptide is produced in the cell bodies and is thought to be converted to a small molecule that is transported along the processes of the bag cells for release (ARCH, 1972). In response to an appropriate stimulus, the bag cells discharge synchronously, causing the hormone to be released into the neurohemal sheath (KUPFERMANN, 1970; KUPFERMANN & KANDEL, 1970; ARCH, 1972; PINSKER & DUDEK, 1977; DUDEK & BLANKENSHIP, 1977). The hormone then appears to cause muscle fibers in the ovotestis to contract, thereby triggering the first step in the release of eggs (COGGESHALL, 1967).

Once fertilized, the ova are packaged in irregular shaped egg cases (or capsules), each containing several ova. The egg cases are strung together and deposited as an egg ribbon or cordon (Figure 1). We have analyzed certain aspects of egg-laying in wild specimens of *Aplysia californica* in the laboratory and have examined the factors that determine the varying number of eggs that are packaged in each egg case by different animals of the species. Two aspects of this problem have often been commented upon (for review see BRIDGES, 1975; SWITZER-DUNLOP & HADFIELD, 1977). First, what accounts for the considerable observed variability in the number of eggs per capsule in the egg masses of different individuals of the same species? Thus SWITZER-DUNLOP & HADFIELD (*op. cit.*) found 5 to 7 eggs per capsule in ***A. dactylomela,***

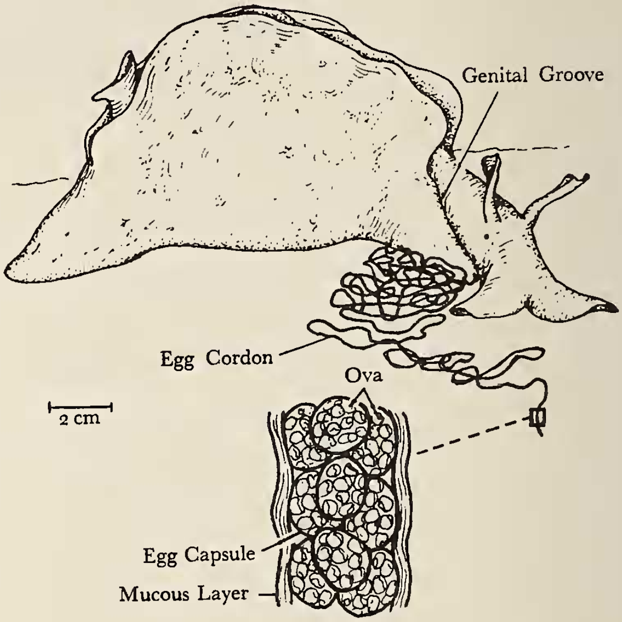

Figure 1

Egg laying in *Aplysia californica*. The adult deposits egg masses consisting of a long string of egg capsules, each containing varying amounts of eggs (after KRIEGSTEIN *et al.*, 1974) while OSTERGAARD (1950) found 7 to 15 eggs per capsule. Are these differences related to age, individual variations or nutritional state of the parent? Second, what accounts for the marked differences in the number of eggs per capsule between the various species of *Aplysia?* BRIDGES (1975) suggests that among the Aplysiidae an inverse relationship exists between the diameter of the egg and the number of eggs per capsule.

In this paper we have used size of the animal as an index of age and examined the first question. We found that the number of eggs per capsule is directly related to the size (and therefore presumably to the age) of the animal. Small and young animals invariably package fewer eggs per capsule, whereas larger and older animals package many.

METHODS

Wild animals collected off the coast of California were shipped to New York and maintained in 720 L capacity, temperature controlled, recirculating aquaria at 16 to 20° C. Animals were fed a daily portion (about 10% wet body weight) of the algae *Ulva* and *Gracilaria.* No special measures were taken to induce spawning.

Spawn was collected daily, freed of excess water, and weighed. Three lengths of cordon, a minimum of 10 cm, were then measured. The weight of each section was determined as rapidly as possible and the mean weight per centimeter calculated. The mean number of ova per capsule was determined ty triplicate counts on 3 randomly chosen sections (9 observations). The section to be counted was placed on a microscope slide, depressed with a cover slip and counted with a binocular microscope at 256× magnification.

To determine the number of capsules per centimeter a minimum of 3 0.5 cm portions were cut from the cordon and centrifuged at 2 000 rpm (1000×g) for 20 minutes to compact the ova. A squash preparation was made and 0.5 cm portions counted at 160× magnification. The mean number of capsules per centimeter was calculated.

The diameters of cordon and ova were determined with the binocular microscope fitted with an ocular micrometer; a standard procedure of rounding off partial units was adopted. Three cordon portions were cut at random and kept immersed in sea water. Duplicate determinations of the diameter of each portion were made with a binocular microscope at 160× magnification. After the diameters of the cordon were taken, they were each covered with a cover slip, depressed, and the diameters of 3 ova chosen at random and from different capsules were taken at 400× magnification. The mean number of ova per centimeter was the product of the mean number of ova per capsule and the mean number of capsules per centimeter.

Coefficients of correlation (r) and lines of best fit were obtained from a least squares regression using a PDP11 laboratory computer.

RESULTS

Collections of spawn were made from (wild) animals, ranging in size from small (presumably young), weighing as little as 19 g to large (presumably older) animals weighing to 1 300 g. In the course of examining the spawn we found that the mean number of eggs per capsule of cordon ranged from 3 to 93. Because of this remarkably wide range we explored a number of variables to determine which might affect how many eggs are packaged per capsule.

We first examined the relation of number of eggs per capsule to body weight (a crude index of the age of the animals). Three sections of egg mass were examined and the mean number of eggs per capsule determined. In the 78 animals examined a linear and fairly steep correlation was found between animal weight and number of eggs per capsule (Figure 2). This correlation was highly sig-

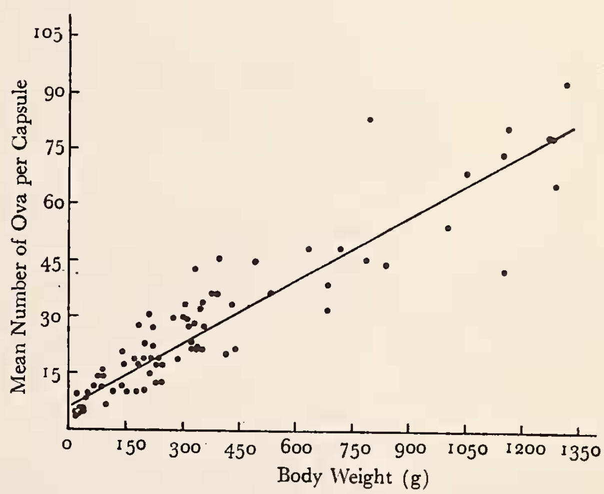

Figure 2

Mean number of ova per capsule as a function of body weight. Animals ranged in weight from 20 g to 1300 g and the ova varied in number from 3 to 93. Each datum point is the mean of 9 observations on 1 animal (see Methods for details). There is a significant correlation ($n=78$; $r=0.92$; $p < 0.01$) between the number of ova per capsule and the body weight nificant: r = 0.92, p < 0.01. Large animals packaged many more ova per capsule than did small ones. A doubling in animal weight led to roughly a two-fold increase in the number of ova per capsule.

To determine whether the increase in eggs per capsule was accompanied by large egg capsules, we next measured the number of capsules per centimeter. We found that for animals weighing between 19 g and about 450 g the numbe of capsules per cm decreased progressively (n = 12, r = 0.84, p < 0.01, b = −0.38). For heavier animals weighing from 450 g to 1 300 g the number of capsules per cm decreased less (n = 43, r = 0.77, p < 0.01, b = −0.06), even though in this range of weights the mean number of ova per capsule doubled (Figure 3A). This suggests that

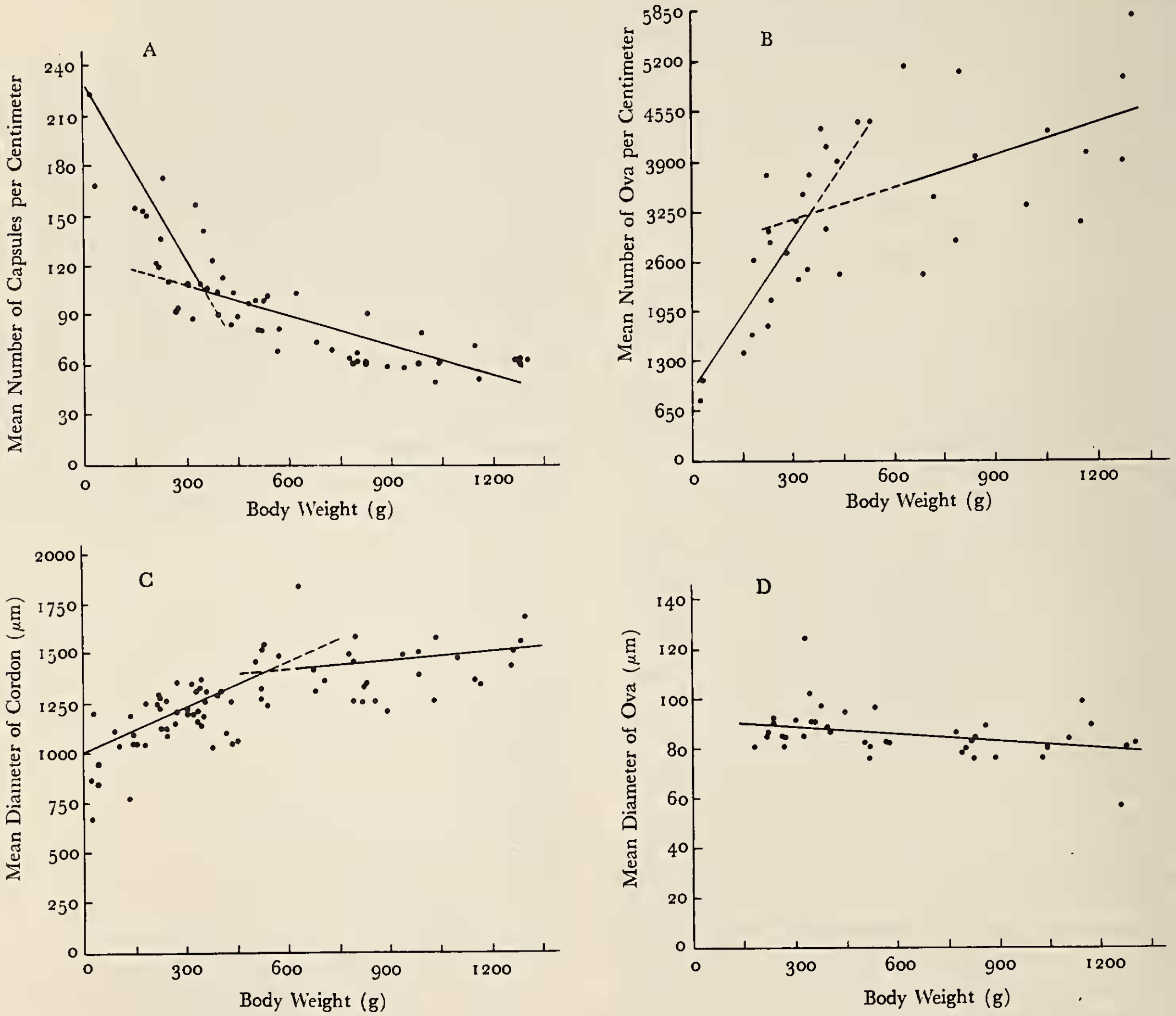

Figure 3

Quantitative aspects of egg packaging

Part A: Mean number of egg capsules per centimeter of egg string as a function of the animal's body weight. Each point is the mean of 3 observations on the same animal. The number of egg capsules per centimeter decreases markedly as the animals grow from 20 g to 400 g (n = 12; r = 0.84; p > 0.01; slope = −0.38); beginning at 400 g, the decrease is less (n = 43; r = 0.77; p < 0.01; slope = −0.06). In this and in all subsequent figures, the dotted lines indicate an extrapolation of the plotted functions

Part B: Mean number of ova per centimeter of egg string as a function of body weight. The number of ova increases throughout the reproductive life of the animal, but during later stages of the life cycle (> 300 g; n = 13; r = 0.33; p = NS) the increase is less than it is early in the life cycle (20 - 300 g; n = 22; r = 0.79; p < 0.01)

Part C: Mean diameter of the egg cordon as a function of the animal's body weight. From 20 g to 600 g (n = 54; r = 0.59; p < 0.01). From 600 g to 1300 g (n = 26; r = 0.09; p = NS)

Part D: Mean diameter of ova as a function of body weight (n = 40; r = 0.38; p < 0.05) up to 400 g the animal seems to compensate for the increase in number of eggs per capsule by increasing the volume of each capsule. Animals heavier than 400 g seem to pack the ova more densely into capsules of similar size. Independent measurements of the number of ova per cm provide some of the support for this argument (Figure 3B).

As a check on this finding we explored 2 other variables: the diameter of the cordon and the diameter of the ova. We found (Figure 3C) that as animals grew up to 500 g the diameter of the cordon increased about 31% presumably indicating an increase in the size of the egg capsules ($n = 54$, $r = 0.59$, $p < 0.1$). Beyond 600 g the diameter did not increase significantly ($n = 26$, $r = 0.09$, $p = ns$). This finding suggests that after a certain point the capsules remain about the same size or at least increase less. Although this finding could simply indicate that the same sized egg capsules are simply packed differently, we also found that the size of the ova decreased slightly (about 13%) as a function of size of the animal (Figure 3D).

Is there a correlation between the weight of the animal and the total number of eggs deposited? To answer this question we examined the weight of the spawn as a function of weight of the animal and found a roughly linear correlation ($n = 64$, $r = 0.70$, $p < 0.01$) between these 2 variables (Figure 4A). Although there was a great deal of variability, small animals tended to lay small amounts of spawn (less than 1 g), large animals tended to produce great amounts of spawn (more than 25 g). When we next compared the relation of spawn as a percent of body weight to body weight (Figure 4B) we found that independent of size, animals produce egg masses that were about 2.1% of their body weight (with a range of 0.22 to 5.9%).

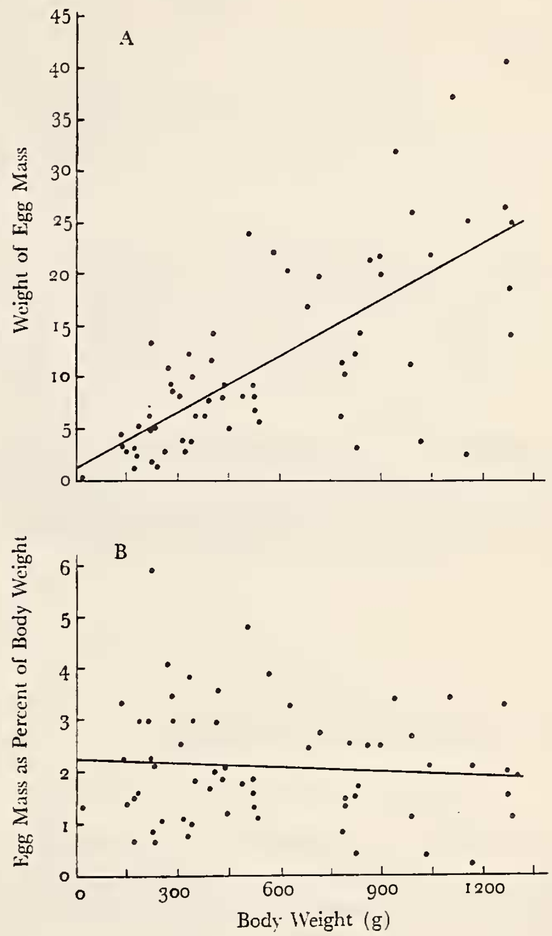

Figure 4

Egg mass and body weight

Part A: Weight of the egg mass as a function of body weight. The egg mass increases progressively from < 1 g to > 25 g as the animals mature ($n = 64$; $r = 0.70$; $p < 0.01$)

Part B: Egg mass as percent of total body weight ($n = 64$; $r = 0.11$; $p = NS$)

DISCUSSION

We have examined the egg laying capability of *Aplysia californica* as a function of age; using weight of animal as a crude index of age (see KRIEGSTEIN *et al.*, 1974; COGGESHALL, 1976). Two key findings emerge from this study: 1) animals produce spawn throughout much of their postmetamorphic life. Animals as small as 19 g (1.5% of maximum body weight in our samples) already produce viable ova; 2) the number of ova packaged per capsule varies systematically with aging.

On the basis of these findings we propose a schema illustrated in Figure 5. As animals grow they package progressively more ova per egg capsule than do smaller animals. At first the increase in ova per capsule is compensated for by an increase in size of the capsule (see middle age animals in Figure 5). However, beyond 600 g the size of the capsule may not keep pace with the increase in the number of ova per capsule, resulting in a relatively tighter package of ova (old animals; Figure 5). The mechanisms that determine the density of packing and the consequences for the ovum of alterations in density are interesting questions that need now to be explored.

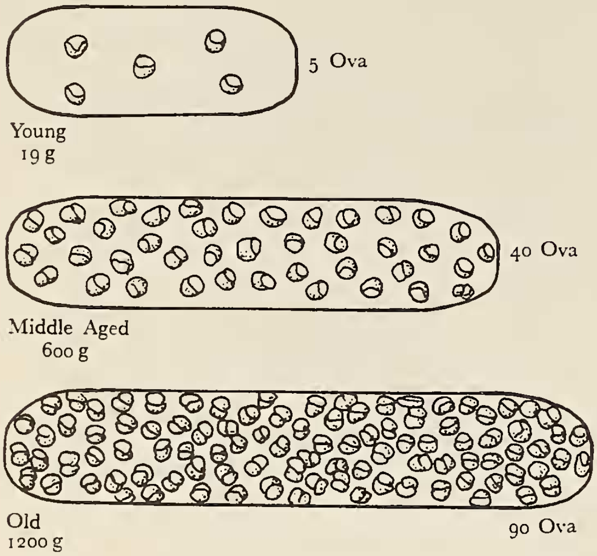

Figure 5

Schematic drawing indicating changes in number of ova per capsule and inferred changes in packing density

Larger animals also lay larger (more) spawn than do smaller animals. This increase in spawn is a reflection of the fact that animals generally lay egg masses of a relatively fixed average percentage – about 2% of their body weight.

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